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Evaluation of sedative and anticonvulsant activities of Unmadnashak Ghrita

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Abstract

'Unmadnashak Ghrita' (UG) is a ayurvedic formulation containing *Ferula narthex* (6 g), *Gardenia gummifera* (6 g), *Ellataria cardamom* (6 g), *Bacopa monneri* (6 g), and cow's ghee (clarified butter fat) (76 g). In the present study, neuropharmacological activities of UG were evaluated for its gross behavioural effect, pentobarbitone sleeping time, spontaneous locomotor activity, antagonism to amphetamine induced hyperlocomotor activity, analgesic activity by tail flick test, rota-rod performance (motor coordination test), maximal electroshock (MES) induced seizures, and pentylenetetrazol (PTZ) induced convulsions in mice. The formulation showed CNS-depressant activity in gross behavioural test, potentiated pentobarbitone sleeping time and there was significant decrease in spontaneous locomotor count in mice. The formulation also antagonized the behavioral effects of CNS-stimulant drug amphetamine, and showed analgesic effect in mice. UG failed to affect the motor coordination test. The formulation also protected mice from MES and PTZ induced convulsions. These results suggest that UG has CNS-depressant and anticonvulsant activity in mice.

Keywords: Anticonvulsants; Ayurveda; Herbal formulation; Sedative; Panchagavya

1. Introduction

'Unmadnashak Ghrita' (UG) is a pachagavya class of ayurvedic formulation. Panchagavya is the term described in Ayurveda to refer collectively or individually the five products obtained from cow (pancha = five and gavya = products obtained from cow) viz. milk, ghee (clarified butter fat), curd, urine and dung. There are large number of formulations mentioned in ancient ayurvedic texts containing panchagavya components either alone, or in combination with other substances of herbal mineral or animal origin for the treatment of several diseases (Shah, 1997). Ghritas are one of the subclass of panchagavya ayurvedic formulations in which cow's ghee is boiled with prescribed 'Kasayas' (decoction of herbal ingredient) and 'Kalkas' (aqueous paste of powdered herbal drugs) according to the formula in ancient texts (Fulzele et al., 2002). Recent literature documents immunomodulatory activity of Haridradi Ghrita (Fulzele et al., 2003), and Ashtamangal Ghrita (Fulzele et al., 2002a) and anti-inflammatory activity of Jatyadi Ghrita (Fulzele et al., 2002b). The components of Unmadnashak Ghrita (UG) are *Ferula narthex* (6 g), *Gardenia gummifera* (6 g), *Ellataria cardamom* (6 g), *Bacopa monneri* (6 g) and cow's ghee (76 g). UG is indicated in ayurveda for the treatment of mania, epilepsy and other disorders of central nervous system (Tripathi, 1994). 'Unmad' is a condition described in ayurvedic sciences which pertains to the disorder of CNS and resembles schizophrenia, mania and excitement. The word 'Nashak' means 'anti', thus unmadnashak means formulation which antagonizes CNS excitement symptoms. One of the herbal ingredient, i.e. *Ellataria cardamom* was reported to posses antioxidant (Nair et al., 1998) activity. The cardamom oil was found to have anti-inflammatory, analgesic and antispasmodic activity (Al-Zuhair et al., 1996). *Bacopa monniera* is a well-known nootropic plant reported to possess sedative (Malhotra and Das, 1959), cognitive enhancer (Nathan et al., 2001; Roodernrys et al., 2002; Stough et al., 2001), broncho-vasodilatory (Channa et al., 2003), hepatoprotective (Sumathy et al., 2001), antidepressant (Sairam et al., 2002), and antiulcer (Sairam et al., 2001) activities. The plant *Bacopa monniera* also showed antistress effect, via modulation of HSp 70 expression, superoxide dismultase and cytochrome p450 inhibitory activity in rat brain

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(Chowdhari et al., 2002) and antioxidant activity (Tripathi et al., 1996). *Bacopa monniera* is reported to play a protective role on morphine-induced brain mitochondrial enzyme status in rats (Sumathy et al., 2002). It is also active against leishmaniasis (Sinha et al., 2002). The plant *Gardenia gummifera* was used traditionally as antispasmodic, expectorant, carminative, anthelmintic, cardiotonic, bronchitis, obesity and against skin diseases (Warrier et al., 1995). Asafoetida increases biliousness and is believed to be useful as laxative, analgesic, anthelmintic, antispasmodic and nervine stimulant. It is also recommended against cardiac disorders, teeth carries, jaundice, hysteria and hysterical affections. (Kirtikar and Basu, 1998).

In the ayurvedic system of medicine polyherbal formulations were frequently prescribed for CNS disorders. In these complex formulations, the plant constituents may not only enhance the activity of compounds or counteract the toxic effect of compounds, from other plants but may also act synergistically with other constituents from the same plants (Melanie-Jayne and Houghton, 2003). Until recently, very little work was carried out on such type of herbal formulations. Therefore, it was thought worthwhile to explore neuropharmacological effects of UG to substantiate the claims made in Ayurveda as well as by traditional healers.

2. Materials and methods

2.1. Animals

Male swiss albino mice weighing 25–30 g were used. The animals were housed in the groups of 5–6 in standard laboratory conditions of temperature (25 ± 2 °C), relative humidity (55 ± 5%), lighting (07:00–19:00 h) with food (Lipton India Ltd., Mumbai, Pellets) and water ad libitum. The animals were transferred to the laboratory atleast 1h before the start of the experiment. The experiments were performed during the light portion (08:00–16:00 h). The institutional animal ethical committee approved the protocol.

2.2. Unmadnashak Ghrita (UG)

The formulation UG was obtained as a gift sample for research from Go-Vigyan Anusandhan Kendra, Nagpur (M.S.) India. The formulation was prepared by an expert ayurvedic practitioner having Master's degree in Ayurveda at Govigyan Anusandhan Kendra, Nagpur. Finely powdered dried gum resin of *Ferula narthex* (6 g), dried leaves of *Gardenia gummifera* (6 g), fruits of *Ellataria cardamom* (6 g) and aerial parts of *Bacopa monneri* (6 g) were mixed thoroughly and to the powdered blend, 192 ml of water (8 parts of the powdered bulk) was added to prepare a paste. Cow's ghee (76 g) was melted in a separate container and the prepared paste was then boiled till water gets evaporated from the preparation. The Ghrita formulation was then allowed to cool and then filtered. The botanical identity of raw material was established by a qualified botanist at Govigyan Anusandhan Kendra, Nagpur. The formulation is used as received in the present study.

2.3. Behavioural effect

The behavioural effect of UG (50 and 100 mg/kg p.o.) was assessed by the method described by Irwin et al. (1968). Briefly, the effects of UG on different behavioural paradigms in animals were scored with the use of nine degrees, that is, with a scale of 0–8. The base score for normal signs or effects is 4, scores below 4 are subnormal responses, those above 4, for supernormal. The base score for abnormal signs is 0, and the maximal score is 8. In the items mentioned below, the base score is given in parentheses. The animals were observed for 2 h after treatment for alertness (4), stereotypy (0), spontaneous locomotor activity (4), reactivity to touch response (4), body position (4), righting reflex (0) and lacrimation (0). The formulation was also fed upto 2 g/kg to the animals and they were observed for 24 h for mortality if any.

2.4. Pentobarbital-induced sleeping time

The animals were divided in five groups ($n = 5$). Group I served as control and received pentobarbitone sodium (45 mg/kg i.p.) only. Groups II, III, IV and V were injected with pentobarbitone sodium (45 mg/kg i.p.) 30 min after oral administration of UG (50, 100, 200 and 300 mg/kg). The time elapsed between loss and recovery of the righting reflex was recorded as sleeping time and recorded for control and pretreated animals.

2.5. Effect of UG on spontaneous locomotor activity

The animals were treated with UG (50, 100, 200 and 300 mg/kg p.o.) and spontaneous locomotor activity was recorded by using Actophotomotor (Centronics, Bombay), wherein interruption of beam of light generated a pulse which was recorded on digital counter. The locomotor count for each animal was recorded for 5 min at 30 min interval for 1.5 h.

2.6. Amphetamine antagonism

Mice were pretreated with UG (100, 200 and 300 mg/kg p.o.) 30 min before amphetamine administration (2 mg/kg i.p.). The motor activity count was recorded for 5 min at 30 min interval for 1.5 h after the injection of stimulant.

2.7. Analgesic activity (tail flick method)

The animals were divided in four groups ($n = 5$). The animals, which withdraws its tail from heat source in 3.0 ± 1.0 s, were preselected 24 h before start of the experiment. Group I animals were treated with vehicle only. Groups II, III and IV animals were fed orally UG, 100, 200 and 300 mg/kg,

respectively. Basal reaction time to radiant heat was noted by placing the tip of the tail on radiant heat source. The tail withdrawn from the heat source was taken as end point. The cut off period of 10 s was taken to prevent the damage to the tail.

2.8. Motor coordination test

The animals were administered orally UG (100, 200 and 300 mg/kg) and effect on motor coordination was evaluated by the method described by Dunham and Miya (1957). The animals were trained on rota rod apparatus to remain for 3 min on rotating rod at the speed of 25 rpm. Twenty four hours later the formulation UG was administered orally to the groups of mice ($n = 5$) in the doses 100, 200 and 300 mg/kg, respectively. The control group received vehicle only. The ability of mice to remain on rotating rod was assessed before and 30 min after the treatment. The fall off time of each animal from rotating rod was noted.

2.9. Maximal electroshock (MES) induced seizures

The animals were divided into five groups ($n = 6$) and treated with either UG (100, 200, 300 and 500 mg/kg p.o.) or saline. They all received current of 42 mA for 0.2 s duration through electroconvulsiometer (Techno, India) using corneal electrodes (Swinyard, 1949) after 60 min of oral administration of test formulation. The incidence and duration of extensor tonus was noted. A complete abolition of hind limb tonic extension was considered as 100% protection.

2.10. Pentylenetetrazol (PTZ) induced seizures

The animals were divided into five groups ($n = 6$) and treated with UG (100, 200, 300 and 500 mg/kg p.o.) or saline. They were all treated with PTZ (70 mg/kg s.c.) 45 min later and observed for occurrence of seizures and presence or absence of clonic convulsions. Animals devoid of seizures were considered protected (Adzu et al., 2002).

2.11. Statistics

The data obtained were analysed using student *t*-test or one-way analysis of variance (ANOVA) followed by Dunnett *t*-test. The level of significance was set at $P < 0.05$.

3. Results

3.1. Behavioural effects

The formulation 'Unmadnashak Ghrita' was found to be safe after oral administration upto the dose of 2 g/kg. No mortality was observed upto this dose up to 24 h. The animals were observed for 2 h after oral administration of test formulation (50 and 100 mg/kg). During the observation period various behavioural scores were noted as shown in Table 1. There was decrease in alertness, spontaneous locomotor activity and reactivity to touch stimuli. The animals did not show loss of righting reflex, and body position was normal and no stereotypy and lacrimation was observed.

Table 1
Behavioural assessment of Unmadnashak Ghrita in mice[a]

Behavioural signs (normal scores)	UG (mg/kg)	
	50	100
Alertness (4)	3.2 ± 0.44	1.2 ± 0.45
Stereotypy (0)	–	–
Spontaneous locomotor activity (4)	2 ± 0.0	1 ± 0.0
Reactivity to touch stimuli (4)	3.4 ± 0.90	2.2 ± 0.44
Body position (4)	4 ± 0.0	4 ± 0.0
Righting reflex (0)	0 ± 0.0	0 ± 0.0
Lacrimation (0)	–	–

[a] $n = 5$ mice pretreated orally with UG (50 and 100 mg/kg) and behavioural signs were noted as mean score of five animals.

3.2. Pentobarbital induced sleeping time

Pretreatment with the formulation UG potentiated pentobarbitone induced sleeping time. UG treatment (50, 100, 200 and 300 mg/kg p.o.) prolonged pentobarbitone sleeping time from 54.2 ± 3.92 min to 77.4 ± 3.69 min, 92.8 ± 4.55 min, 106.2 ± 4.54 min, 129.6 ± 7.098 min, respectively ($P < 0.05$) (Table 2). The effect thus seems dose dependant.

3.3. Effect of UG on spontaneous locomotor activity

UG treated rats exhibited significant decrease in spontaneous locomotor activity ($P < 0.005$). The observations are given in Fig. 1.

3.4. Amphetamine antagonism

Hyperlocomotor activity induced by CNS-stimulant drug amphetamine was significantly antagonized by UG treatment (200 and 300 mg/kg p.o.) ($P < 0.001$). The observations are recorded in Fig. 2.

Table 2
Effect of UG on pentobarbitone sleeping time in mice[a]

Sr. No.	Treatment	Mean sleeping time ± S.E.M. (min)
1	Control (pentobarbitone sodium 45 mg/kg, i.p.)	54.2 ± 3.92
2	UG (50 mg/kg p.o.)	77.4 ± 3.69*
3	UG (100 mg/kg p.o.)	92.8 ± 4.55#
4	UG (200 mg/kg p.o.)	106.2 ± 4.54#
5	UG (300 mg/kg p.o.)	129.6 ± 7.098#

All animals were treated with pentobarbitone sodium (45 mg/kg, i.p.) 30 min after oral administration of either vehicle or UG.

[a] $n = 5$

* $P < 0.05$ compared with control group (student *t*-test).

# $P < 0.001$ compared with control group (student *t*-test).

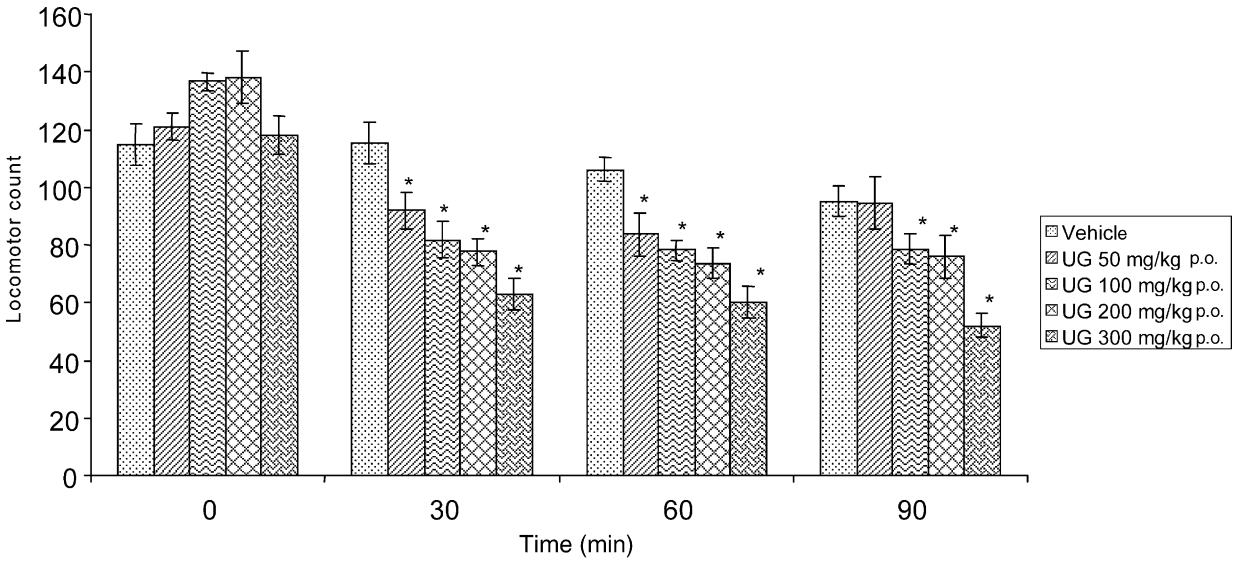

Fig. 1. Effect of UG on spontaneous locomotor activity in mice ($n = 5$; $^{*}P < 0.005$, compared with vehicle; statistics ANOVA one-way followed by Dunnett *t*-test). Mice were pretreated with either vehicle or UG 50, 100, 200 and 300 mg/kg p.o.

3.5. Analgesic activity

Pretreatment with test formulation UG showed a significant increase in pain threshold in mice ($P < 0.05$). The results are shown in Fig. 3.

3.6. Motor coordination test

The animals treated with UG did not fall off from the rotating rod and remained on rod at least for 3 min. The formulation UG did not induce any motor incoordination in the doses tested. (data not shown).

3.7. Maximal electroshock (MES) induced seizures

The formulation UG exhibited almost dose dependent anticonvulsant activity. UG significantly decreased the duration of tonic extensor phase in MES induced seizures. The observations are given in Table 3.

3.8. Pentylenetetrazol (PTZ) induced seizures

UG significantly delayed the onset of tonic and tonic clonic hind limb extensor phase ($P < 0.01$) in PTZ induced convulsions in mice. The observations are given in Table 4.

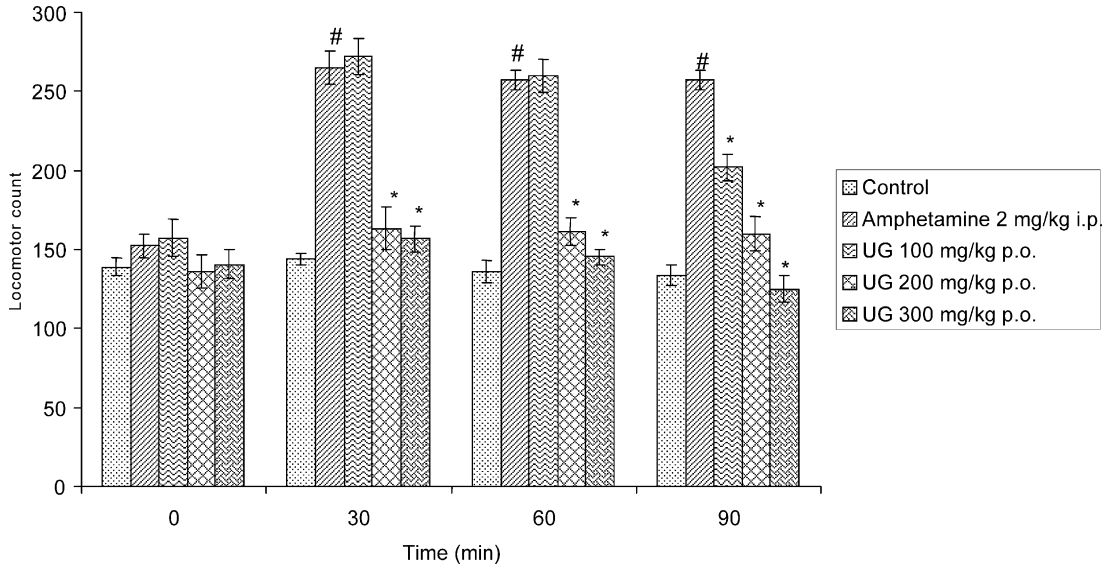

Fig. 2. Effect of UG on amphetamine stimulated locomotor activity in mice ($n = 5$; $^{\#}P < 0.001$ compared with vehicle; statistics ANOVA one-way followed by Dunnett *t*-test).$^{*}P < 0.001$ compared with amphetamine (statistics ANOVA one-way followed by Dunnett *t*-test.) Animals were pretreated with either vehicle or UG 100, 200 and 300 mg/kg or vehicle orally, 30 min before amphetamine 2 mg/kg i.p. treatment.

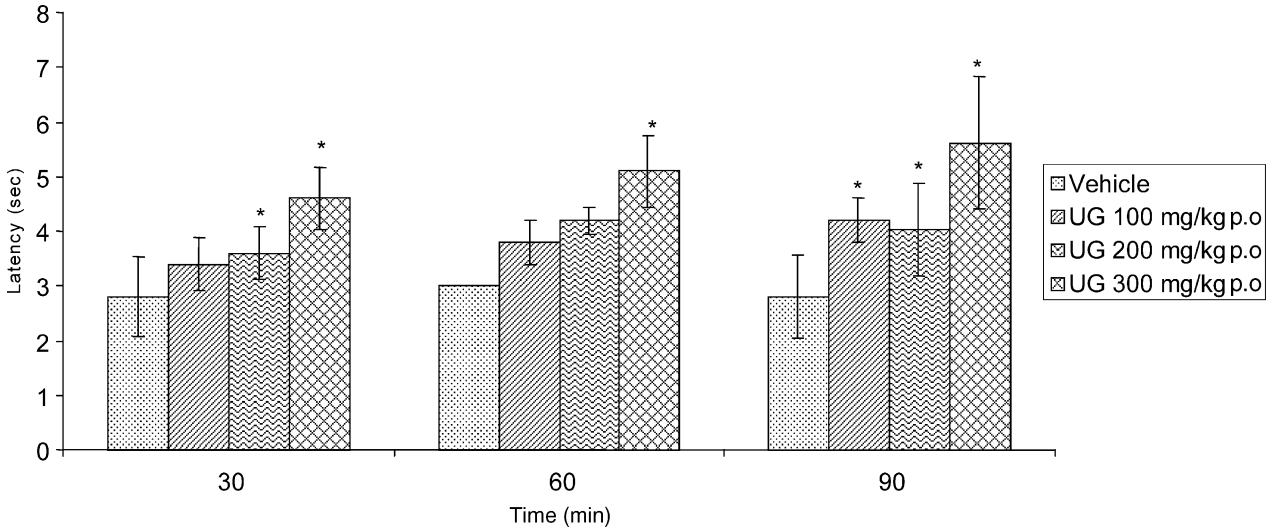

Fig. 3. Analgesic effect of UG (tail flick method) in mice ($n = 5$; $^{*}P < 0.01$, compared with vehicle; statistics used is one-way ANOVA followed by Dunnett *t*-test).

Table 3
Effect of UG on MES induced seizures in mice[a]

Sr. No.	Treatment	Duration of tonic ± S.D.	Incidence of convulsions
1	Vehicle	16.5 ± 2.99	6/6
2	UG 100	14.5 ± 1.29	6/6
3	UG 200	12.11 ± 0.86	6/6
4	UG 300	7.8 ± 2.18*	5/6
5	UG 500	4 ± 0.82*	3/6

[a] $n = 6$.
* $P < 0.01$ compared with vehicle (student *t*-test).

Table 4
Effect of UG on pentylenetetrazol-induced seizures in mice[a]

Sr. No.	Treatment	Time (s)	
		Duration of tonic ± S.D.	Tonic clonic with hind limb extension ± S.D.
1	Vehicle	44.85 ± 7.79	93.66 ± 7.23
2	UG 100	52.95 ± 4.65	96.1 ± 7.998
3	UG 200	64.56 ± 6.45*	116.16 ± 9.23#
4	UG 300	77.05 ± 7.145*	162.2 ± 15.56#
5	UG 500	102.5 ± 10.52*	196.67 ± 3.4#

Values are mean ± S.D. Mice were pretreated with vehicle or UG orally 60 min before the injection of pentylenetetrazol.
[a] $n = 6$.
* $P < 0.01$ compared with vehicle (one-way ANOVA followed by Dunnet *t*-test).
# $P < 0.01$ compared with vehicle (one-way ANOVA followed by Dunnet *t*-test).

4. Discussion

The present study demonstrates the neuropharmacological effect of polyherbal formulation Unmadnashak Ghrita (UG) in mice. In the preliminary behavioural assessment, UG exhibited decreased locomotor activity, reduced alertness and altered response to external touch stimuli. However, UG did not cause muscle weakness, or abnormal body posture and stereotypy at a dose that markedly decreased the locomotor activity. The results reveal CNS-depressant effects of UG in mice. The above activity was corroborated by potentiation of pentobarbitone sleeping time by UG. It is well known that potentiation of pentobarbitone sleeping time is due to sedative/or hypnotic activity attributed to involvement of central mechanisms involved in the regulation of sleep (N'Gouemo et al., 1994). The UG also induced decrease in spontaneous locomotor activity and antagonized the hyperlocomoter activity induced by CNS-stimulant drug amphetamine which is suggestive of depression (Pal and Dandiya, 1993). The analgesic activity showed by UG in tail flick test may be attributed to increase in the central inhibitory neurotransmitter GABA, since GABA is known to induce antinociception. (Ali et al., 1995). It seems probable that the UG did not act through peripheral neuromuscular blockage, but rather it evoked its action through centrally located mechanism centrally and thus acts as a CNS-depressant (Capasso et al., 1996). UG also inhibited MES induced seizures and PTZ induced seizures in mice. This may suggest that the anticonvulsant action of UG is mediated by the channel of GABA/benzodiazepine receptor complex. The formulation may act by increasing GABA concentration in brain because PTZ is a known $GABA_A$ receptor antagonist (Kasture et al., 2000). Ghritas are lipophilic formulations. In our laboratory it was found that the log octanol–water partition coefficient; log $P_{o/w}$ of Unmadnashak Ghrita is 2.33. Bodor and Buchwald (2003) reported that majority of CNS-active therapeutic agents have a log $P_{o/w}$ value between 0.5 and 5.5 and they also reported log $P_{o/w}$ for antiepileptic drugs (1.34 ± 1.34, $n = 53$) and antipsychotic (4.27 ± 1.20, $n = 64$). In the designing of new CNS-active drugs, the chances of

success are more if the calculated log $P_{o/w}$ is around 2.0 (Hansch et al., 1987). Thus, log $P_{o/w}$ value of UG supports its CNS action. One of the ingredients of UG, *Bacopa monneri* is a well-known ayurvedic nootropic plant, Singh and Dhawan (1997) reported that bacosides (mixture of dammarane-type triterpenoid saponins) improved the acquisition, consolidation and retention in various models of behavioural responses in albino rats. The data regarding use of constituents of *Bacopa monneri* for epilepsy and mental insufficiency and illness is not available. The data regarding the CNS activity of other individual herbal drugs is also scant in modern literature. Therefore, at this stage it is difficult to corroborate the present findings with the role of plant constituent present in the test formulation. However, further studies will be directed to characterize the role of individual plant constituent, and to elaborate exact mechanism of action of the formulation. In conclusion, this study provides evidence supporting traditional use of ayurvedic polyherbal formulation 'Unmadnashak Ghrita' as CNS-depressant and anticonvulsant agent, and suggests that the formulation may be a useful therapeutic tool in epilepsy and conditions pertaining to mania.

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